IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 9891
SNYDER *et al.*	Art Unit: 1639
Application No.: 09/849,781	Examiner: WESSENDORF, Teresa D.
Filed: May 4, 2001	Atty. Docket: 2681.0030002/RWE/JKM
For: **Protein Chips for High Throughput Screening of Protein Activity**	

Declaration of Barry Schweitzer, Ph.D. Under 37 C.F.R. § 1.132

Attn: Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

The undersigned, Barry Schweitzer, residing at 459 Maple Avenue, Cheshire, CT, USA, declares and states as follows:

1. I am currently employed by Life Technologies Inc. (hereinafter "LTI"), a licensee of the above-captioned application. I hold the positions of Director of Integrated Technologies, Molecular Biology Systems Division. My credentials are provided in the curriculum vitae that is attached to this declaration as Exhibit A. I received my Ph.D. degree in Pharmacology from Yale University. As seen from my attached curriculum vitae, I have published many papers related to protein microarrays. Based on my education and experience, I am an expert in the field of yeast and human genomics, proteomics, and molecular genetics.

2. I have reviewed and am familiar with U.S. Application No. 09/849,781, (hereinafter "the '781 application") filed on May 4, 2001, the Office Action dated July 7, 2009 ("the Office Action"), issued by the U.S. Patent and Trademark Office in the

present application, and the currently pending claims, filed in the Reply to Office Action with this declaration.

3. The '781 application presently claims a positionally addressable array comprising a plurality of different substances on a solid support, with each different substance being at a different position on the solid support, wherein the density of the different substances on the solid support is at least 100 different substances per cm^2, and wherein the plurality of different substances comprises 61 purified active kinases or functional kinase domains thereof of a mammal, 61 purified active kinases or functional kinase domains thereof of a yeast, or 61 purified active kinases or functional kinase domains thereof of a Drosophila.

4. In the Office Action at pages 7-12, the Examiner asserts that the claims are allegedly not enabled. Specifically, the Examiner asserts that the specification does not enable the claimed array comprising 61 kinases and functional kinase domains from a mammal, yeast or Drosophila.

5. In making this declaration, it is my opinion that at the time this application was filed, an ordinary practitioner in the field of genomics and proteomics would have been able to make and use the presently claimed positionally addressable arrays based on knowledge available to those in the field in combination with the detailed disclosure of the '781 application. It is also my opinion that any experimentation required for making and using the presently claimed positionally addressable arrays would have been routine and thus not inordinate or excessive.

6. As discussed in detail below, the specification of the '781 application clearly provides sufficient disclosure for a typical practitioner in the field of proteomics to make and use positionally addressable arrays comprising 61 purified active kinases or functional kinase domains thereof of a mammal, 61 purified active kinases or functional kinase domains thereof of a yeast, or 61 purified active kinases or functional kinase domains thereof of a Drosophila. Based upon this disclosure, in combination with what was known at the time of filing of the present application, the use of kinases from various organisms, including mammals and Drosophila, in the preparation of the presently claimed positionally addressable arrays, would not have required undue experimentation, but rather, routine and straightforward experiments.

8. Protein kinases and functional kinase domains used in the positionally addressable arrays that form the basis of the present claims were, at the time this application was filed, all well-known, and well-characterized. *See* Hunter and Plowman, "The protein kinases of budding yeast: six score and more," *TIBS 22*:18-22 (1997) at page 18, first column, first paragraph (cited in Applicants' 6th SIDS submitted on April 20, 2009). It was also well recognized at the time of filing of this application that kinases are highly conserved such that homologs exist between yeast and many other organisms. *See* Manning *et al.*, "The Protein Kinase Complement of the Human Genome," *Science 298*:1912-1934 (2002) at page 1913, first column, first paragraph (cited in Applicants' 6th SIDS submitted on April 20, 2009). Furthermore, the regulation of the different kinases and the phosphorylation motifs of substrates recognized by related kinases are often the same, indicating that they behave similarly biochemically. *See id.* Furthermore, as function is often highly conserved, human kinases can be

substituted for yeast kinases, illustrating the highly conservative nature of these proteins. *See* Lee and Nurse, "Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*," *Nature 327*: 31-35 (1987) (cited in Applicants' 6th SIDS submitted on April 20, 2009). Therefore, at the time this application was filed, the "state of the art" in protein kinases was such that a practitioner possessing a typical level of skill in proteomics, such skill including but not limited to gene cloning, protein expression and purification and analysis, would have readily recognized from the '781 application, and the knowledge available in the art, that kinases of yeast, mammals and Drosophila could routinely be utilized to practice the presently claimed invention.

9. The Examples set forth in the '781 application describe positionally addressable protein arrays made with purified, active kinases isolated from yeast. The specification describes that the purified, active yeast kinases were prepared by cloning yeast kinase genes into a high copy URA3 expression vector. *See* the '781 application at page 28, lines 28-30. The plasmids containing the vector sequences were transformed into yeast, and *Ura+* colonies were selected. Plasmids were rescued in *E. coli*, then transformed into the pep4 yeast strain for kinase protein purification. *See id.* at page 28 line 36 to page 29 line 7. Purified, active kinases were attached to polydimethylsiloxane (PDMS) chips, and the chips comprising the purified, active yeast kinases were assayed for the phosphorylation of 17 different substrates to determine *in vitro* kinase activity. *See id.* at page 33 line 14 to page 34 line 19. *See id.* at page 29 line 26 to page 30 line 21.

10. In 2000, while working for Molecular Staging, developing antibody based arrays, I read Dr. Michael Snyder's paper entitled "Analysis of yeast protein kinases

using protein chips." *See* Zhu et al., "Analysis of yeast protein kinases using protein chips," *Nature Genetics 26*: 283-289 (2000). Dr. Snyder's paper described work that was extremely impressive and unexpected. Dr. Snyder's discoveries, in fact, motivated me to accept a position at Protometrix, Branford, CT, a protein array company that had licensed Dr. Snyder's technology. When I joined Protometrix, work was already underway to use the information set forth in the '781 application, and known in the art at the time regarding protein kinases and their highly conserved homology between yeast and many other organisms, to develop protein arrays with active human kinases.

11. Using the information set forth in the '781 application and the known homologies between human and yeast kinases, researchers at Protometrix were able to informatically identify genes for human kinases and utilize algorithms to identify many kinase functional domains. The genes were cloned into a recombinant bacmid (baculovirus shuttle vector), transfected into Sf9 insect cells, and cultured in 96 well plates. *See* Protein-Protein Interaction Profiling on Invitrogen ProtoArray™ High-Density Protein Microarrays, Application Note, Invitrogen page 2, column 2, paragraph 3 (2005) (hereinafter "Protein-Protein Interaction Profiling," Exhibit B). In many cases, efforts were made to clone the full length protein kinases as well as the kinase active catalytic domains. All the proteins were expressed as N-terminal glutathione-S-transferase (GST) fusion proteins. *See id.* page 2, column 1, paragraph 1.

12. After a growth period, the cells were harvested and lysed under nondenaturing conditions as in the '781 application. *See* Protein-Protein Interaction Profiling at page 2, column 3, paragraph 1. *See* the '781 application at page 29, line 8-16. The lysates were further loaded and eluted off of glutathione resin in 96 well

plates under nondenaturing conditions. *See* Protein-Protein Interaction Profiling at page 2, column 3, paragraph 1. Over 90% of protein kinases expressed and purified using the methods described in the '781 application were active as demonstrated by catalytic activity including autophosphoylation, wherein a protein kinase phosphorylates itself. *See id.* In contrast, only approximately 10% of protein kinases were active when other methods known at the time were utilized, including the use of high throughput methods with kinase expression in E. coli.

13. Finally, using the purified kinases I have described, Protometrix developed a positionally addressable array comprising at least 100 different proteins on a solid support, with each different protein being at a different position on the solid support, wherein the density of the different proteins on the solid support was at least 100 different proteins per cm^2, and contained at least 61 purified active human kinases or functional kinase domains, as presently claimed in the '781 application. Protometrix's technology, sold as Invitrogen's Human ProtoArray High Density Protein Microarrays™, are manufactured with thousands of different quality controlled recombinant human proteins and contain approximately 400 active human kinases and functional kinase domains. *See* B. Schweitzer *et al.*, Development and Validation of Kinase Substrate Screening on Human ProtoArray High Density Protein Microarrays™, Invitrogen, Inc., page 2, column 1, paragraph 2 to page 3, column 1, paragraph 1 (2004) (hereinafter "Schweitzer") (Exhibit C). *See also* Access to the Human Proteome on a Microarray Scale, Invitrogen, Inc., Tables 1 & 2 (2007) (hereinafter "Access to Human Proteome") (Exhibit D). Several commercial versions of this array have been sold with between 1,500 to 9,000 human proteins. The activity of the arrayed kinases has been verified,

including demonstrated catalytic activity by incubating the arrays with radioactive ATP and measuring autophosphorylation. *See* Schweitzer, page 2, column 2, paragraph 1 to page 3, column 1, paragraph 1.

14. It is my opinion that the '781 application provides a clear disclosure of how to make and use the presently claimed positionally addressable arrays. Those working in the field of proteomics at the time this application was filed were aware that yeast, mammalian and Drosophilia kinases are highly conserved and homologous, and therefore that the teachings of purified, active yeast kinase arrays in the '781 Application could be used to routinely prepare similar arrays with mammalian and Drosophilia kinases. Equipped with this information, researchers at Protometrix, using the information disclosed in the '781 application, were able to develop protein arrays comprising human kinases. *See*, e.g., the '781 application at pages 25-35. Thus, in my opinion, a typical practitioner in the field of proteomics would consider the production of an array using at least 61 purified, functional kinases from yeast as detailed in the '781 application, to also allow for the routine production and use of arrays comprising purified, active kinase and functional kinases domains from other organisms, including mammals and Drosophila, as set forth in the presently claimed invention.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Barry Schweitzer, Ph.D.

Date: March 2, 2010

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